

Determination of forest soil organic nitrogen determination using technique of X-ray absorption near-edge structure

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Abstract: The forest soil organic N was investigated using N K-edge X-ray absorption near-edge structure (XANES) to gain an insight into the relationship between N speciation and its transformation in quantity. Soil samples were collected from spruce, hemlock and pine forests in central Taiwan. Results showed that various organic N types could be revealed by XANES spectra. Amide and pyrrolic N are the major parts of the composition in the humic substance, soluble organic nitrogen and original soils. The relative distribution of N speciation differed in treatments and vegetations samples. The spruce had a significant difference from the hemlock in soluble organic nitrogen (SON) spectra at 402.3 eV energy peak. In the A-horizon soils, the relative amount of pyridinic N was much higher than that in the O-horizon soils, indicating N transformation in quantity in the mineral horizon was different from that in the organic horizon, which might play an important role in N cycling of forest ecosystems.

Keywords: Forest soils; N K-edge XANES spectra; Nitrogen speciation Organic nitrogen; Nitrogen transformation

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Introduction

Soil nitrogen is one of the most important nutrients for plant growth. Almost 95% of total N in soil is present as organic nitrogen compounds (Mengel 1996). However, plants mainly uptake N in inorganic forms, such as NH_4^+ and NO_3^- , from soils. Therefore, the mineralization of soil organic N, a process of organic N transforming into inorganic N, is of importance in natural forest ecosystems without artificial management. As reported previously, many factors can influence the process of soil N mineralization, of which soil organic N is one of the most important factor that differs greatly in various ecosystems.

Due to the difficulty of determination on complex soil organic N, there is more than 50% of total soil organic N still unknown. As reviewed by Schulten and Schnitzer (1998), there is a possible distribution of the different types of N forms in soils as follows: 40% of proteinaceous materials, 5%–6% of amino sugars, 35% of heterocyclic N compounds; and 19% of NH_3 . This suggests that proteinaceous materials and heterocyclic N account for the most portion of total N in soils. However, this portion of N is difficulty in identification and determination, because they have no simple unit structure, they consist of a variety of units with widely different structures, and the units are assembled randomly (Vairavamurthy *et al.* 1997). In traditional wet-chemistry methods for organic N speciation in soils, the material is treated with hot mineral acids/bases to liberate nitrogenous constituents, thereby primarily identifying the amino compounds; these compounds have been grouped as acid-insoluble N, NH_3 -N, amino acid N, amino sugar N, and hydrolysable unknown N. Using

pyrolysis-gas chromatography, Schulten *et al.* (1997) suggested that the unknown fraction from soils contains largely heterocyclic N compounds, such as pyridines and pyrroles. However, there is concern in the pyrolysis method about the artificial formation of heterocyclics at high temperatures through dissociation/rearrangement of the original structures. On the other hand, nondestructive ^{15}N NMR spectroscopy revealed mainly amide N in organic matter from soils. The main criticism about the NMR method is its inadequate sensitivity due to the low abundance of ^{15}N in natural organic matter. The inability to detect heterocyclics in the soils' organic matter was thought to be due to this limitation. Another nondestructive method, X-ray photoelectron spectroscopy (XPS), was applied for N speciation in fossil fuels and some marine sediments; however, this technique suffers mainly from inadequate spectral resolution of the different chemical forms of nitrogen.

Nitrogen K-edge XANES spectroscopy is a selective, sensitive and nondestructive method for N speciation determination. Preliminarily revealed and reviewed by Vairavamurthy and Wang (2002), the characteristic energies of some major types of organic N compounds are assigned. However, the energies studied by Jokic *et al.* (2004) were differed by approximately 1.2 eV compared with the results of Vairavamurthy and Wang (2002). The discrepancy between the two reports was the energy calibration and it was eliminated by re-calibrated reference positions by Jokic *et al.* (2004).

With typical fitting of XANES spectra, the relative distribution of the different types of N forms in soils and standard humic/fulvic acid could be calculated as a percentage of the sum total using the areas corresponding to the different peaks identified in fitting. Therefore, the relationship between soil organic N form/quantity and soil mineralization rate of forests can be established according to the results from XANES. The objective of this study is to investigate the type and quantity of soil organic N using N-K edge XANES to better understand the relationship between organic N and N mineralization in forest soils of central Taiwan.

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Methods and Materials

Samples preparation

Soil samples were collected from different vegetations, including spruce, hemlock forests and a meadow at Tatachia site, at central Taiwan (23°28' N, 120°52' E). O- and A-horizon soils were collected under various vegetations. Soil sample preparation was made as following treatments:

- (1) Soil samples were air-dried then ground to pass 300-mesh sieve.
- (2) Air-dried soil samples were extracted by 0.2-M NaOH, centrifuged, then acidified by 2 -M HCl to separate humic substance and fulvic acid fractions.
- (3) Using soil/water ratio of 1:5, soil soluble organic N (SON) was extracted. Solid SON was obtained from the extracts after freeze-dried.
- (4) Standard humic substance and fulvic acids were ordered from IHSS, International Humic Substances Society.
- (5) Standard organic N reagent in the assay included glutamic acid, glycine, guanine and glucosamine.

N K-edge XANES analysis

The N-XANES spectra were collected at the 11A1 Dragon Beamline at the National Synchrotron Radiation Research Center, Tsinchu, Taiwan. The beamline was operated at the storage ring of 1.5 GeV and a current of 120-200 mA using the spherical grating monochromator with a 1200 groove/mm. The photon energy scan ranged from 395 to 420 eV (Schwarzkopf *et al.* 1999). All measurements were made at room temperature and under high vacuum (10^{-9} torr) to minimize contamination and disturbance from the air (Banereft 1992).

Results and discussion

Glycine and glutamic acid have the same N functional group as $R-NH_2$ that was characterized at the peak position of 403.5 eV (Fig. 1A). Glucosamine has the H_2N-B functional group that occurred at the peak position of 402.5 eV. Guanine has more complex N functional groups than the former chemicals, which had the characteristic peaks at 396.8, 398.0 and 403.0 eV. Compared with previous reports, the characteristic energy positions of various N functional groups were not consistent. As reviewed by Vairavmurthy and Wang (2002), characteristic energies peaks of the largest transitions for pyridine, oxidized pyridine derivatives, pyridone, pyrrole, amide/peptide and nitro compound are at 399.8, 401.4, 401.9, 403.2, 402.5 and 404.8 eV, respectively. While Jokic *et al.* (2004) calibrated their references positions for peak assignments and characterized as pyridine (398.6 eV), pyridone (400.7 eV), amide (401.3 eV) and pyrrolic (402.0 eV) moieties.

An important factor affecting the energy level of a π^* transition is the effective nuclear charge of the absorbing nitrogen atom. This level increases with increasing effective nuclear charge or decreasing electron density on the nitrogen atom. This effect is clearly shown by the difference in energies between the pyrrolic and the pyridinic forms, both representing conjugated unsaturated systems. Pyrrolic nitrogen has a peak at a higher energy level than pyridinic nitrogen does, mainly because of lower electron density on the nitrogen site. The major reason for this effect is the sharing of the lone pair of electrons on the ni-

trogen site in the B-cloud of the conjugated five-membered pyrrolic ring. Because they are not shared similarly in a six-membered pyridine ring, the pyridinic nitrogen site carries a higher electron density, accordingly, the π^* resonance peak is at a lower energy level. Compared nitrogen atom in the chemical structure, therefore, the associated energy shift of organic nitrogen substance can be identified in the XANES spectra.

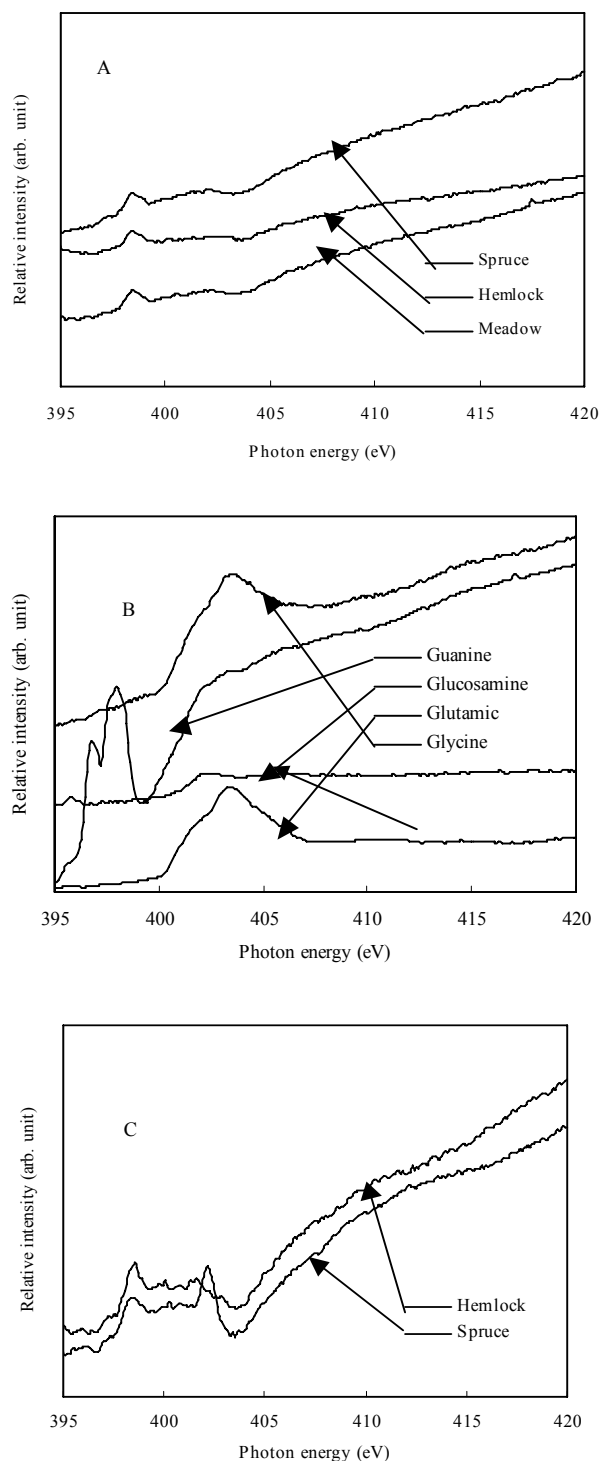


Fig. 1 N K-edge XANES spectra of various organic standard chemicals (A), humic substances (B) and soluble organic nitrogen (C)

Due to the limitation of experimental time, some samples only were selected to analyze their N K-edge XANES spectra. As depicted in Fig. 1, the humic substances extracted by NaOH in hemlock, spruce and meadow soils showed no significant difference (Fig. 1B), and the similarities were in the original spruce and hemlock soils. The water soluble organic N from the hemlock and spruce soils showed significant difference at the energy position of 402.2 eV which occurred in the spruce forest but not in the hemlock forest (Fig. 1C). Because all of a sample's compositions are exposed to the beam simultaneously, its spectrum represents the sum of their spectral contributions. Thus, the quantitative information on a sample's chemical composition can be obtained by appropriately deconvoluting its spectrum. Fig. 2 showed the deconvoluted results of various humic substance spectra obtained from hemlock (A), spruce (B) and meadow (C) soils, respectively, with manual normalization. According to the peak area, the relatively various N distributions were present at Fig. 3. It was obvious that no significant difference of extracted humic substances existed among the hemlock, spruce and meadow soils. The peak position of 403.1 eV accounted for almost 80% of all samples, which might represent nitrogen in amide/peptide or pyrrolic forms.

Similarly, the relative N distributions, in the soluble organic form and original soils were calculated and shown in Table 1. All samples showed that the main distribution occurred at the energy peak of 403.2 eV, representing amide and pyrrolic N. The SON of spruce soil was obviously different from that of the hemlock soil. The spruce SON spectrum had an apparent peak at 402.3 eV while no such a peak was observed at the spectrum of hemlock SON sample. Relatively in the soil samples, O-horizons had higher content in amide/pyrrolic forms than A-horizons, but no significant difference was present between the hemlock and spruce soils. Organic N in the A-horizons are generally derived from the O-horizon in forest ecosystems. The results suggested that pyridinic N were mainly produced in the mineral soil.

Table 1. The relative distribution of various N forms in soluble organics and soils (%)

Functional group		Photon energy (eV)			
		396.1	398.3	402.3	403.2
		-	Pyridine	Pyridone	Amide/Pyrrole
SON	Hemlock	8.24	11.06	0.00	80.70
	Spruce	7.56	11.79	8.96	71.68
Soil N	Hemlock O	3.88	10.54	0.00	85.58
	Hemlock A	9.39	21.48	0.00	69.13
	Spruce O	3.79	10.82	0.00	85.39
	Spruce A	0.00	32.93	0.00	67.06

However, the assignments of N in this study can not be matched with the other reports, thus, further energy calibration of the XANES spectra would be strongly helpful to identify the soil organic N and correlate to its transformation in forest ecosystems.

Conclusions

Synchronized radiation is developed to be an effective multi-disciplinary research tool. But its application in forest science is limited. Using N K-edge XANES technique, forest soil organic nitrogen can be highlighted in the element. More details

of the composition and structure of the soil organic N were revealed from the spectra. Amide/pyrrole is the most important composition of the forest soil organic N illustrated by the XANES. In the spruce and hemlock soils, the amide/pyrrole N accounted for almost 85% of the total soil N. But a significant difference was present between the SONs of the spruce and hemlock soils that extracted by water. However, the relationship between various N speciation and its transformation in quantity merits further research.

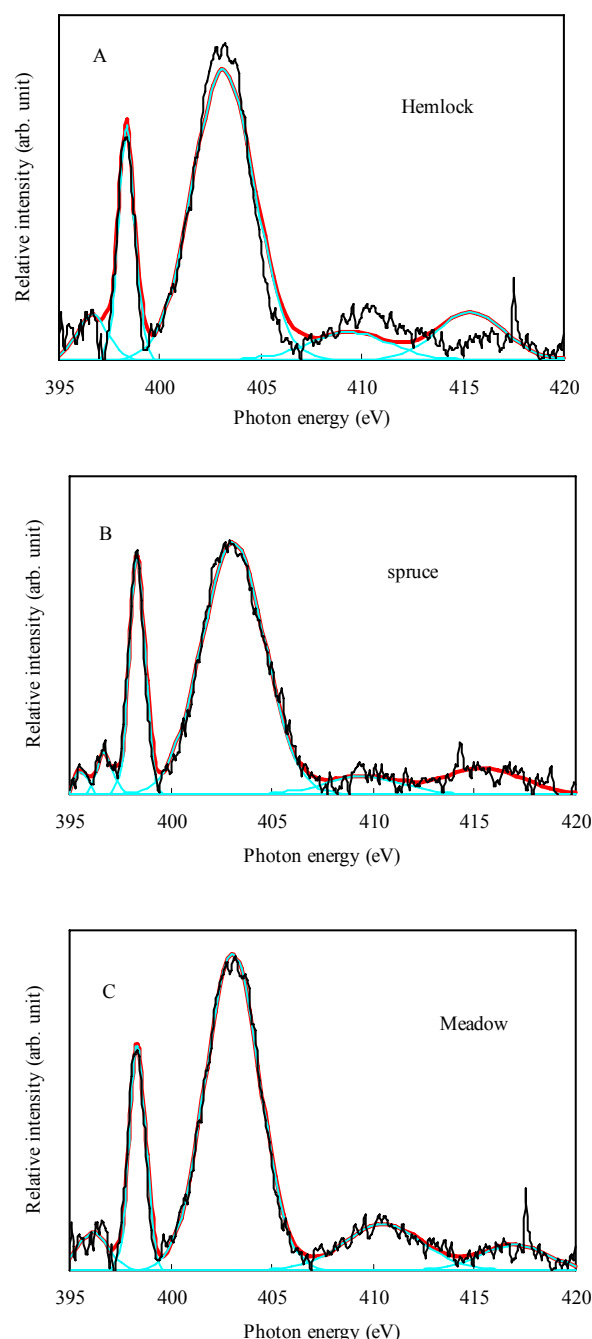


Fig. 2 Gaussian fitting of XANES spectra for various humic substances from hemlock (A), spruce (B) and meadow soil (C). (The blue line represents the various peak fitting, the red line represents the final fitting result, and the black line represents the observed result)

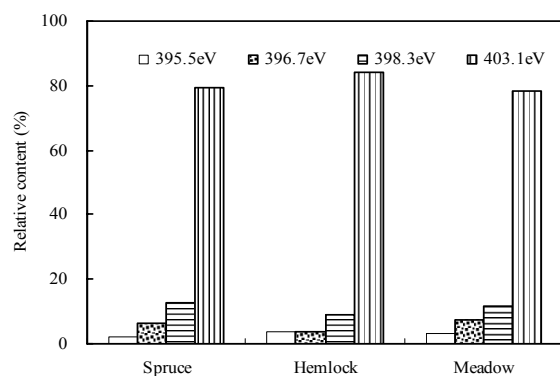


Fig. 3 Histograms of the relative distribution of the different types of organic nitrogen forms in humic substances

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